CLAIMS

1. A recombinant protein whose essential constituent polypeptide sequence is:

- either that of a 19 kilodalton (p19) C-terminal fragment of the surface protein 1 of the merozoite form (MSP-1 protein) of a *Plasmodium* type parasite other than *Plasmodium vivax* which is infectious for mammals, in particular for man, the C-terminal fragment remaining normally anchored to the parasite surface at the end of its penetration phase into human erythrocytes in the event of an infectious cycle;
- or that of a portion of that fragment which is also capable of inducing an immune response which can inhibit *in vivo* parasitemia due to the corresponding parasite;
- or that of a peptide which is capable of inducing a cellular and/or humoral immunological response equivalent to that produced by said p19 fragment or said portion of that fragment; and

said recombinant protein possibly further comprising conformational epitopes which are unstable in a reducing medium and which constitute the majority of the epitopes recognised by human antiserums formed against the corresponding *Plasmodium*.

2. A recombinant protein according to claim 1, characterized in that it is recognised by human antiserums formed against the corresponding *Plasmodium* or against a homologous *Plasmodium* when it is in its non reduced state or in a reduced non irreversible state, but is not recognised or is only recognised to a slight extent by these same antiserums when it is irreversibly reduced.

3. A protein according to claim 1 or claim 2, characterized in that it inhibits the reactivity of an immune human antiserum with the corresponding p42 produced under the same conditions, also the

p42 itself and in that the p42 can only partially inhibit the reactivity of said human antiserum against the p19.

4. A recombinant protein according to any one of claims 1 to 3, characterized in that it is essentially deprived of any polypeptide sequence normally upstream of the C-terminal polypeptide sequence of the p33 (33 kDa N-terminal fragment) normally on the side associated with the p19 in the corresponding p42, before natural cleavage of the latter, said last C-terminal polypeptide sequence of the p33 containing less than 50 amino acid residues when it is present.

5. A recombinant protein according to any one of claims 1 to 3, characterized in that it is essentially deprived of any polypeptide sequence normally upstream of the C-terminal polypeptide sequence of the p33 (33 kDa N-terminal fragment) normally associated with the p19 in the corresponding p42 before natural cleavage of the latter, said last C-terminal polypeptide sequence of the p33 containing less than 10 amino acid residues when it is present.

6. A recombinant protein according to any one of claims 1 to 3, characterized in that it is essentially deprived of any polypeptide sequence normally upstream of the C-terminal polypeptide sequence of the p33 (33 kDa N-terminal fragment) normally associated with the p19 in the corresponding p42 before natural cleavage of the latter, said last C-terminal polypeptide sequence of the p33 being limited, when it is present, to that which retains a substantial degree of conservation in *Plasmodium* which are infectious for man, such as *P. falciparum* or *P. vivax*.

7. A recombinant protein according to any one of claims 1 to 6, characterized in that the portion of p19 fragment contains at least one of the two EGF regions normally contained in this p19.

8. A recombinant protein according to any one of claims 1 to 7, characterized in that the molecular weight of said p19 fragment or of said portion of p19 fragment is in the range 10 to 25 kDa, in particular in the range 10 to 15 kDa.

9. A recombinant protein according to any one of claims 1 to 8, characterized in that it also comprises a glycosylphosphatidylinositol (GPI) group of the type enabling the p19 fragment to anchor to the host cell, in particular a eukaryote cell, preferably a cell of an insect infectable by a baculovirus, in which said recombinant protein is expressed.

10. A recombinant protein according to any one of claims 1 to 8, characterized in that it is deprived of the extremely hydrophobic C-terminal portion which intervenes in induction of anchoring of said recombinant protein to the cell membrane of the host in which it is expressed, in particular in a eukaryote cell, preferably a cell of an insect infectable by a baculovirus.

11. A recombinant protein according to claim 10, characterized in that it is hydrosoluble.

12. A recombinant protein according to any one of claims 1 to 11, characterized in that it contains the p19 sequence of the MSP-1 protein of *Plasmodium falciparum* or said portion of the corresponding fragment.

13. A recombinant protein according to any one of claims 1 to 12, characterized in that it contains the p19 sequence of the MSP-1 protein of *Plasmodium cynomolgi* or said portion of the corresponding fragment.

14. An oligomer of the recombinant protein according to any one of claims 1 to 13.

15. An oligomer according to claim 14, characterized in that it contains 2 to 50 monomer units of said polypeptide sequence of the recombinant protein as defined in any one of claims 1 to 13.

16. A recombinant protein according to any one of claims 1 to 14, characterized in that it is conjugated to a carrier molecule for use in the production of vaccines.

17. A vaccination composition against a *Plasmodium* type parasite which is infectious for man, containing as an active principle a recombinant protein whose essential constituent polypeptide sequence is:

- either that of a 19 kilodalton (p19) C-terminal fragment of the surface protein 1 of the merozoite form (MSP-1 protein) of a *Plasmodium* type parasite which is infectious for man, said C-terminal fragment remaining normally anchored to the parasite surface at the end of its penetration phase into human erythrocytes in the event of an infectious cycle;
- or that of a portion of that fragment which is also capable of inducing an immune response which can inhibit *in vivo* parasitemia due to the corresponding parasite;
- or that of a peptide which is capable of inducing a cellular and/or humoral immunological response equivalent to that produced by said p19 fragment or said portion of that fragment; and

said recombinant protein further comprising conformational epitopes which are unstable in a reducing medium and which constitute the majority of the epitopes recognised by human antiserums formed against the corresponding *Plasmodium.*

18. A vaccinating composition according to claim 17, characterized in that its active principle consists of a recombinant protein according to any one of claims 2 to 13 or 16, or an oligomer according to claim 14 or claim 15.

19. A vaccinating composition against a *Plasmodium* type parasite which is infectious for man, containing as an active principle an oligomer of a recombinant protein according to claim 17 or claim 18.

20. An antibody specifically recognising the p19 of a MSP-1 protein of the merozoite form of a *Plasmodium* type parasite which is infectious for man other than *Plasmodium vivax* and which does not recognise *Plasmodium vivax*.

21. An antibody according to claim 20, characterized in that it is monoclonal.

22. An antibody according to claim 20, characterized in that it is monoclonal and in that it specifically recognises the p19 of *P. falciparum*.

23. A monoclonal antibody according to claim 20, characterized in that it specifically recognises the p19 of *P. vivax*.

24. A differential diagnostic process to distinguish between a parasitic infection due to *P. vivax* and a parasitic infection due to another *Plasmodium*, characterized by bringing a biological sample infected with *Plasmodium* into contact with an antibody according to claim 23 and with an antibody according to claim 21 or claim 22, and detecting the production or non-production of an immunological reaction depending on the case.

25. A recombinant baculovirus type modified vector containing, under the control of a promoter contained in the vector and able to be recognised by cells transfectable by said vector, a first nucleotide sequence coding for a signal peptide which is compatible with expression in a baculovirus system, characterized by a second sequence downstream of the first, also under the control of said promoter and coding for the peptide sequence:

- either of a 19 kilodalton (p19) C-terminal fragment of the surface protein 1 of the merozoite form (MSP-1 protein) of a *Plasmodium* type parasite which is infectious for man, the C-terminal fragment remaining normally anchored to the parasite surface at the end of

its penetration phase into human erythrocytes in the event of an infectious cycle;

- or of a portion of that peptide fragment provided that the expression product from the second sequence in a baculovirus system is also capable of inducing an immune response which can inhibit *in vivo* parasitemia due to the corresponding parasite;
- or of a peptide which is capable of inducing a cellular and/or humoral immunological response equivalent to that produced by said peptide fragment p19 or said peptide fragment portion; and

said nucleotide sequence also having a G and C content in the range 40% to 60%, preferably at least 50%, of the totality of nucleotides from which it is constituted.

26. A modified vector according to claim 25, characterized in that the said second polypeptide sequence is in accordance with that defined in any one of claims 2 to 13.

27. A modified vector according to claim 25, characterized in that the second nucleotide sequence is a synthetic sequence.

28. A modified vector according to any one of claims 25 to 27, characterized in that the first nucleotide sequence codes for a signal peptide from *Plasmodium vivax* and normally associated with the *Plasmodium* MSP-1 protein.

29. A modified vector according to any one of claims 25 to 28, characterized in that the second nucleotide sequence is deprived at its 3' terminal end of the hydrophobic C-terminal end sequence which is implicated in induction of anchoring said recombinant protein to the cell membrane of the host in which it is expressed, in particular in a cell of an insect infectable by a baculovirus.

30. A modified vector according to any one of claims 25 to 29, characterized in that it consists of a modified baculovirus.

31. An organism, in particular an Sf9 type insect cell, transfectable and transfected by the modified vector according to any one of claims 25 to 29.

32. A synthetic DNA containing a first nucleotide sequence for which at least a portion codes for the peptide sequence:

- either of a 19 kilodalton (p19) C-terminal fragment of the surface protein 1 of the merozoite form (MSP-1 protein) of *Plasmodium falciparum*, said C-terminal fragment remaining normally anchored to the parasite surface at the end of its penetration phase into human erythrocytes in the event of an infectious cycle;
- or of a portion of that peptide fragment provided that the expression product of said DNA in a baculovirus system is also capable of inducing an immune response which can inhibit *in vivo* parasitemia due to the corresponding parasite;
- or a peptide capable of inducing a cellular and/or humoral type immunological response equivalent to that produced by said p19 peptide fragment or said portion of that fragment; and

said nucleotide sequence also having a G and C nucleotide content in the range 40% to 60%, preferably at least 50%, of the totality of nucleotides from which said synthetic DNA is constituted.

33. A synthetic DNA sequence according to claim 32, characterized in that its first nucleotide sequence is deprived at its 3' terminal end of the sequence coding for the hydrophobic C-terminal end region normally implicated in inducing anchoring of the p19 protein to the cell membrane of the host in which it is expressed, in particular in a cell of an insect infectable by a baculovirus.

34. A synthetic DNA sequence according to claim 32 or claim 33, characterized in that the first nucleotide sequence is preceded by a signal nucleotide sequence coding for a signal peptide normally

associated with a *Plasmodium* MSP-1 protein, homologous or heterologous relative to the principal sequence.

35. A synthetic DNA sequence according to claim 34, characterized in that the signal sequence originates from *P. vivax*.

36. A synthetic DNA according to any one of claims 32 to 35, characterized in that said first nucleotide sequence includes a 3'-terminal sequence coding for a polypeptide cell membrane anchoring region, said anchoring region fixing the expressed recombinant protein to the surface of the membrane of the host cell transformed with a vector containing said synthetic DNA, said 3' sequence being homologous to that of the principal nucleotide sequence, or heterologous, in particular that from *P. vivax*.

37. A synthetic DNA according to claim 36, characterized in that the 3'-terminal sequence originates from *P. vivax*.

38. A synthetic DNA sequence according to any one of claims 32 to 36, characterized in that it is deprived of said 3'-terminal sequence.

39. A baculovirus type vector according to claim 25, characterized in that it is selected from:

- the virus deposited at the CNCM [Collection Nationale de Cultures de Microorganismes; National Collection of Microorganism Cultures] with registration number I-1659;
- the virus deposited at the CNCM with registration number I-1660;
- the virus deposited at the CNCM with registration number I-1661;
- the virus deposited at the CNCM with registration number I-1662;
- the virus deposited at the CNCM with registration number I-1663.

40. A hybridoma secreting monoclonal antibodies having the specifications of the antibodies of any one of claims 21 to 23.

41. A process for separating a p19 peptide with a given specificity from a mixture of peptides, characterized by bringing said peptide mixture into contact with a corresponding antibody, in accordance with any

one of claims 20 to 23, preferably already fixed on an insoluble support, by subsequently dissociating the antigen-antibody compound formed and by recovering the purified p19 peptide.

42. Use of a protein according to any one of claims 1 to 13 or of the oligomer according to claim 15 or 16 to prepare an immunogen composition which can induce an immune response against a *Plasmodium* infection.

43. A vaccine composition comprising, as active principles, a mixture of a protein according to any one of claims 1 to 13 and either the corresponding p42, with if necessary the least conserved portions deleted, or another recombinant p19 or p42 type protein, originating from a parasite homologous with that from which said protein originates.

44. A vaccine composition according to claim 23, characterized in that the mixture of active principles is selected from the following mixtures:

- *P. falciparum* p19 and *P. vivax* p19;
- *P. falciparum* p19 and *P. falciparum* p42;
- *P. vivax* p19 and *P. vivax* p42, if necessary deprived of its most hypervariable regions;
- *P. falciparum* p19 and *P. falciparum* p42, if necessary deprived of its most hypervariable regions and *P. vivax* p19 and *P. vivax* p42, if necessary deprived of its most hypervariable regions.

45. A hybridoma according to claim 40, characterized in that it has been deposited at the CNCM, (Paris, France) with registration number I-1846, on the 14th February 1997.

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